Comparison of two different procedures for quantification of drugs of abuse in postmortem brain samples

Reiter, Birgit; Stimpfl, Thomas; Holm, Karen Marie Dollerup; Skov, Louise

Publication date:
2013

Document version
Peer reviewed version

Comparison of two different procedures for quantification of drugs of abuse in postmortem brain samples

Birgit Reiter *, Thomas Stimpfl, Medical University of Vienna, Clinical Institute of Laboratory Medicine, Forensic Toxicology, Waehringer Guertel 18-20, 1090 Vienna, Austria

Karen M. Dollerup Holm, Louise Skov, Section of Forensic Chemistry, Department of Forensic Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Frederik V's Vej 11, 2100 Copenhagen, Denmark

* birgit.reiter@meduniwien.ac.at

Introduction

Routine analyses of brain samples are rarely done in the forensic field even though the brain is the site of action for a large number of drugs identified in postmortem cases. The major reason for this is the extensive need for sample preparation and extraction and - in the past - issues about reproducibility of quantitative results have been reported.
New developments in automated extraction procedures should overcome these difficulties.
The aim of this study was to compare a routine method for qualitative and quantitative analysis of body-fluids and tissue samples developed in Vienna to a routine method developed for blood used in Copenhagen.
No optimization was performed beforehand on the Copenhagen method to accommodate for the use of brain tissue.

Methods

Comparative analyses and quantification of eight drugs of abuse (methadone, morphine, amfetamine, benzoylecgonine, cocaine, codeine, diazepam and 7-aminoflunitrazepam) in 19 brain homogenates were conducted. To ensure that at least one drug was represented in all samples case selection was based on the presence of methadone since it is the most frequent drug in Denmark and in this way a thorough statistical evaluation can be performed. In addition the chosen cases showed a wide range of other drugs with different physico-chemical properties.
Deuterated internal standards (IS) were added prior to extraction in both sample preparations. Figure 1 shows the differences in sample preparation, extraction and analyses.

Figure 1.
Comparison of methods for the determination of drugs of abuse in brain samples in Vienna and Copenhagen, resp.

As can be seen in Figure 1 the two methods differed in sample amounts, applied SPE column and analysis technique. The eluates were evaporated in both methods followed by reconstitution in different derivatization solvents for the GC-MS method (Vienna) and reconstitution in the mobile phase for the UPLC-MS-MS method (Copenhagen).

Both methods were partially validated: validation of the Vienna method is presented at TIAFT 2013, for the Copenhagen method matrix effect, SPE recovery, and precision were tested at one concentration level.

Results

Methadone, morphine, amfetamine, benzoylecgonine, cocaine, codeine, diazepam and 7-aminoflunitrazepam were quantified with the deuterated standards. Quantitative results below 10ng/g were not included in the comparison.
Because methadone could be found in every sample, a Deming regression analysis was performed and displayed in Figure 2. A correlation coefficient of 0,977 was found. Morphine, cocaine, codeine and diazepam were found in 12, 11, 6 and 12 samples, respectively. Correlation coefficients for these compounds are shown in Figures 3, 4, 5 and 6. and lie between 0,9972 and 0,9998.

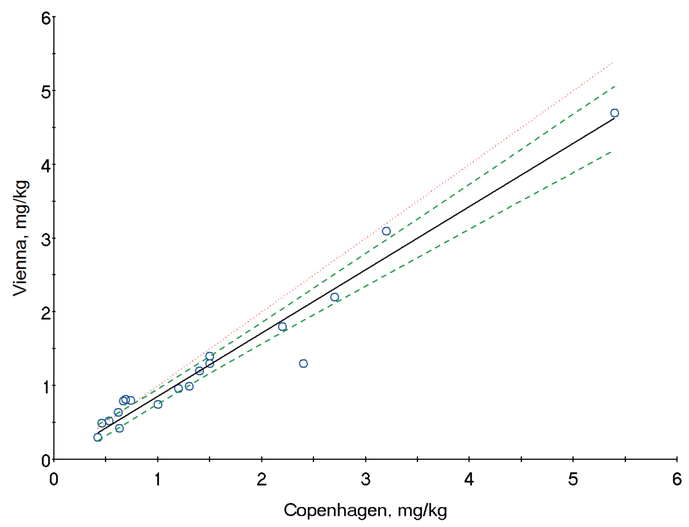

Figure 2.
Vienna vs. Copenhagen method: A Deming regression plot for methadone, n=19, Correlation coefficient 0,977.

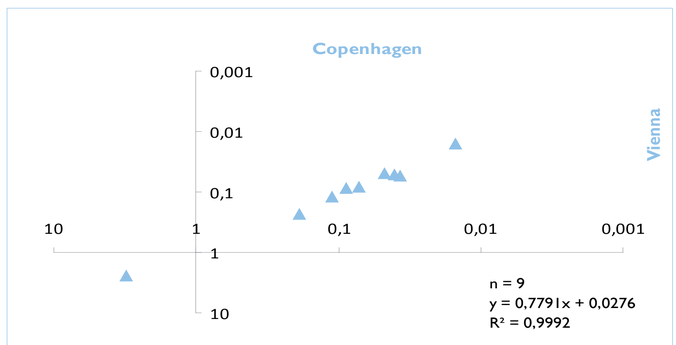

Figure 3.
Comparison of quantitative results of morphine

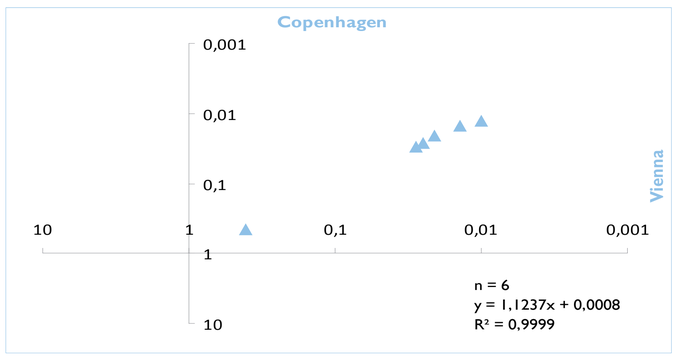

Figure 4. Comparison of quantitative results of codeine

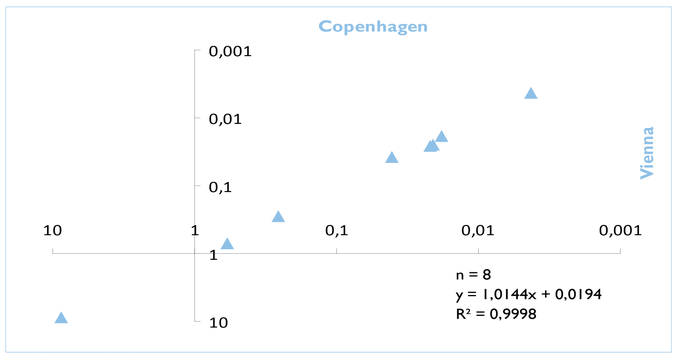

Figure 5. Comparison of quantitative results of cocaine

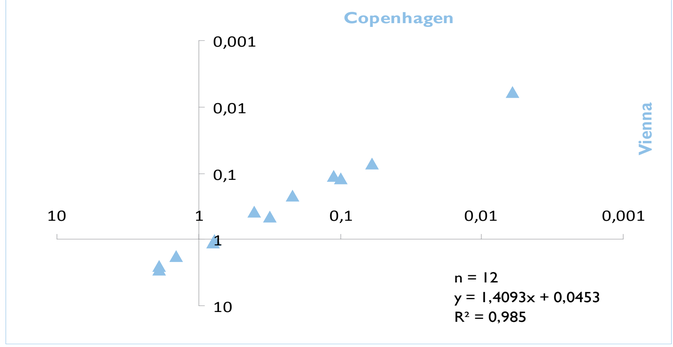

Figure 6. Comparison of quantitative results of benzoylecgonine

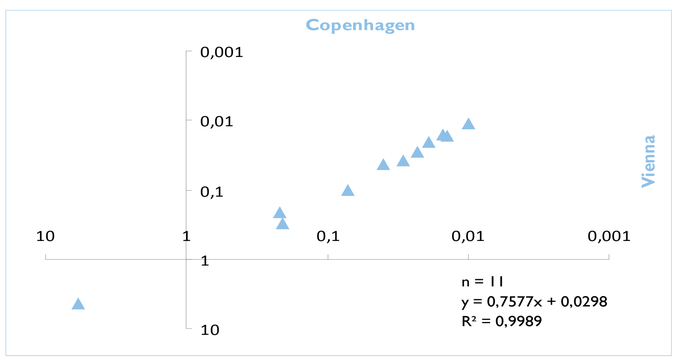

Figure 7. Comparison of quantitative results of diazepam

7-aminoflunitrazepam could only be found in one sample. Because the compound is known for being unstable and is formed from flunitrazepam during storage, the RSD (100%) can not be used. Amfetamine was present in two samples and the RSDs were 5 and 16%, respectively.

Conclusions

The method in Copenhagen had not been modified to accommodate analyses of complex brain tissue and the good correlation for the examined drugs proved that already existing multi-compound SPE methods can be applied to brain samples with reliable quantitative results, if some general considerations are made (e.g. dilution, adaption of capacity). The good correlation between the two methods proves that they are both applicable for quantification of brain samples with reliable results. This is, to our knowledge, the first time a comparison study has been conducted for brain tissue between two laboratories.